Original Research

Diagnostic Value of Soluble Adhesion Molecule Kinetics in Patients with Suspected Myocardial Ischemia Undergoing Dobutamine Stress Echocardiography

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Introduction: Previous studies have shown an exercise-induced increase in circulating adhesion molecules (sICAM-1 and sVCAM-1) in patients with coronary artery disease (CAD). The aim of this study was to evaluate the diagnostic role of changes in serum adhesion molecules in the setting of a dobutamine stress echocardiogram (DSE).

Methods: Thirty patients (18 men and 12 women aged 63.3 ± 10.67 years) with suspected myocardial ischemia underwent a DSE in our department's laboratory of echocardiography in order to identify inducible ischemia. Dobutamine was infused in incremental doses from 5 μg/kg/min up to 40 μg/kg/min. Blood samples were drawn at baseline as well as at peak stress and circulating adhesion molecules sVCAM-1 and sICAM-1 levels were measured by ELISA. Patients with a positive DSE underwent coronary arteriography within 2 weeks of the DSE study.

Results: Sixteen patients had a positive DSE for inducible ischemia while 14 had a negative test. Among the patients with positive DSE, 12 had angiographically significant CAD as well as statistically significantly higher levels of sICAM-1 than DSE negative patients (n=14), both at baseline (302.57 ± 43.37 vs. 267.47 ± 28.03 ng/mL, p=0.028) and at peak stress (322.07 ± 49.64 vs. 260.43 ± 36.45 ng/mL, p=0.001). A significant increase from baseline to peak stress was also noted in this group (from 302.57 ± 43.37 to 322.07 ± 49.64 ng/mL, p=0.043). There were no statistically significant differences in the levels of sVCAM-1 between groups at baseline and there was no change from baseline to peak stress.

Conclusion: Plasma levels of sICAM-1 were found to be elevated in subjects with a positive DSE and angiographically significant CAD compared to patients with a negative DSE, both before and after inducible ischemia. In contrast, no changes were noted regarding sVCAM-1 levels.

Atherosclerosis is currently thought to be strongly associated with chronic inflammatory processes.[1] Inflammation requires transmigration of leukocytes from circulation to the tissue cells, with cellular adhesion molecules mediating the margination, adhesion and transendothelial migration of circulating mononuclear cells from the bloodstream to the extravascular compartment. These molecules seem to play an important role in the progression of atherosclerotic plaque.[2,3]

Intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), expressed by leuko-

cytes and platelets as endothelial integrin ligands, mediate the adhesion and migration of leukocytes in endothelium and are seldom expressed in non-atherosclerotic coronary arteries.[4] Both ICAM-1 and VCAM-1 are present within atherosclerotic lesions,[5] and elevated levels of soluble forms of these molecules may play a role in plaque disruption.[6,7]

Elevated soluble ICAM-1 (sICAM-1) levels have been shown to predict the development of occlusive cardiovascular events in otherwise healthy individuals,[8,9] while both sICAM-1 and soluble VCAM-1 (sVCAM-1) appear elevated among patients with known coronary disease who are at risk for subsequent vascular occlusion.[10-13]

Although elevated levels of sICAM-1 and sVCAM-1 have been shown to predict the risk of major adverse cardiac events,[13,14] and could thus function as prognostic biomarkers, data are limited regarding the association between these molecules and inducible ischemia in the setting of a diagnostic stress test. The purpose of this study was to investigate the impact of dobutamine stress echocardiography (DSE) on plasma concentrations of the endothelial adhesion molecules sICAM-1 and sVCAM-1 in patients with suspected coronary artery disease.

Methods

Study population and protocol

A total of 30 consecutive patients of both sexes (mean age 63.3 ± 10.67, 18 males), who were referred for a DSE study in order to identify inducible ischemia, were enrolled in this study. Patients were referred for DSE because they were unable to undergo a treadmill stress test or after a non-diagnostic examination. A detailed medical history was obtained and any previous history of coronary artery disease, myocardial infarction (MI), percutaneous coronary intervention (PCI), or coronary artery bypass grafting (CABG) was recorded, as well as the presence of coronary disease risk factors such as age, diabetes mellitus, hypertension, hyperlipidemia and smoking. Blood samples were drawn immediately before dobutamine infusion (baseline) and at peak exercise, and sICAM-1 and sVCAM-1 were measured. After the DSE was completed, all patients with inducible ischemia underwent coronary angiography within two weeks.

This study was reviewed and approved by the Bioethics committee of our institution and all patients provided written informed consent.

Echocardiography

Left ventricular dimensions, wall thickness and left ventricular ejection fraction (LVEF) as well as left atrial dimensions were measured according to standard techniques.[15,16]

Diastolic function was evaluated using transmitral flow variables (E, A, DT) and tissue Doppler imaging of the mitral annulus (S, E′, A′, E/E′). Qualitative evaluation of LV systolic function was based on the division of the LV into 16 segments, according to the proposed model by the American Society of Echocardiography.[17] Each segment was characterized as normal, hypokinetic, akinetic or dyskinetic. A segment was considered to be hypokinetic or akinetic when endocardial excursion was <5 mm and <2 mm, respectively.[18]

Stress echocardiography was performed in the standard views using a dobutamine-atropine infusion.[18] Dobutamine was infused in incremental doses of 5, 10, 20, 30 and 40 μg/kg/min in 3-minute stages. The target heart rate was 85% of the predicted maximal heart rate according to age. If target heart rate was not achieved after completing the fifth stage of the DSE study, atropine (1-2 mg) was administered intravenously. Dobutamine infusion was stopped when one of the following criteria was met: maximal heart rate >85% of the predicted maximal heart rate according to age, new appearing wall motion abnormalities, arterial blood pressure elevation to >240/120 mmHg or fall to <90 mmHg, sustained ventricular tachycardia, or ECG changes indicative of myocardial ischemia with coexisting abnormal myocardial thickening or appearance of angina. Beta-blockers, diltiazem, and verapamil were discontinued 3 days before the DSE study.

Myocardial ischemia was documented by the presence of either an ischemic or a biphasic response of a previous normal or hypokinetic/akinetic segment, respectively. Reduced systolic thickening of a previous hypokinetic segment and absence of hyperdynamic response in a segment with previously normal thickening in response to dobutamine infusion were also considered indicative of myocardial ischemia.

All echo studies were performed by the same operator and with the same equipment and were then digitized and stored for further analysis by two independent experienced cardiologists. All echo measurements were considered as mean values of the measurements performed by the two cardiologists separately. The interobserver variability of the two car-

diologists was <5% and the intraobserver variability was <4% for both. In cases of discordance between the two cardiologists, a common final decision was taken after a joint review.

Blood samples

Peripheral venous blood samples were collected before (baseline) and at peak exercise. sICAM-1 and sVCAM-1 levels were measured with a commercial ELISA kit (British Biotechnology Products, Oxford UK) using dual monoclonal antibody two-site ELISAs. Briefly, microtiter ELISA plates were coated overnight with a specific capture antibody (BBIG-I_1 for ICAM-1 and BBIG-V_4 for VCAM-1) at a final concentration of 10 μg/mL in 0.1 M bicarbonate buffer (pH=8.9). Standards and samples were added to the plate, incubated for 2 hours at room temperature and the bound soluble adhesion molecule of interest was detected by sequential incubation with a specific biotin-labeled antibody, followed by horseradish peroxidase-conjugated streptavidin and finally tetramethylbenzidine. The assays were standardized using a recombinant soluble form of adhesion molecule lacking their transmembrane and cytoplasmic domains.

Statistical analysis

Continuous variables are expressed as mean value ± standard deviation, while categorical variables are given as number and percentage. Differences between continuous variables were tested using the independent samples t-test or Wilcoxon rank sum test, as appropriate, and categorical variables using the χ^2 test. Changes from baseline to peak stress were tested by paired sample t-test or Mann–Whitney U test, depending on whether the variables followed a normal distribution or not, respectively. The level of significance was p=0.05.

Results

Sixteen patients had a positive DSE test for inducible ischemia and 14 had a negative test. Among those with a positive DSE the coronary angiogram that followed proved that 12 patients (75%) suffered from CAD, defined as a >50% lumen stenosis in one or more major coronary arteries. These patients (Group A) were compared with those who had a negative DSE (Group B).There were no significant differences in sex, age, weight or risk factors between the two groups. The baseline echocardiographic findings were also comparable. The basic characteristics of the patients with and without inducible ischemia after DSE are summarized in Table 1.

Baseline levels of sICAM-1 were higher in the group of patients with a positive DSE (group A: 302.57 ± 43.37 vs. 267.47 ± 28.03 ng/mL; p=0.028), whereas baseline levels of sVCAM-1 were similar between the two groups. At peak stress, levels of sICAM-1 remained higher in group A compared to group B (322.07 ± 49.64 vs. 260.43 ± 36.45 ng/mL; p=0.001). Moreover, there was a statistically significant increase in sICAM-1 levels in group A from baseline to peak stress (from 302.57 ± 43.37 to 322.07 ± 49.64; p=0.043). In group B there was no statistically significant change from baseline to peak stress. There was no statistically significant correlation between sICAM-1 levels and wall motion score index or time to recovery of wall motion abnormalities.

In contrast, s-VCAM-1 levels remained unchanged from baseline to peak stress in both groups (Table 2).

Discussion

This study showed that, in patients with a positive DSE, plasma levels of sICAM-1 were significantly higher, not only at baseline but also at peak stress, compared to patients with a negative DSE; there was also a trend for sICAM-1 levels to increase from baseline to peak stress. These results are in concordance with the results of the study by Lu Hui-he et al, who reported increased levels of sICAM-1 in patients with stable angina compared to controls.[14] Similarly, Siminiak et al, in a previous study, indicated an increase in plasma levels of sICAM-1 in patients with ischemic heart disease, during chest pain of ischemic origin.[11] Soluble ICAM-1 elevates rapidly after the onset of myocardial ischemia, probably because of increased shedding from endothelial cells, as has been suggested by the detection of increased sICAM-1 levels in the coronary sinus as a result of myocardial ischemia caused by balloon inflation during coronary angioplasty.[19,20]

Another study by Siminiak et al, which evaluated the effects of dipyridamole stress test in combination with radionuclide perfusion imaging on plasma levels of soluble ICAM-1 and VCAM-1 in patients with ischemic heart disease and patients with syndrome X, reported that, after infusion of dipyridam-

Table 1. Basic characteristics of patients with and without inducible ischemia during dobutamine stress echocardiography.

Variable	Group A DSE(+) & CAD(+)	Group B DSE(-)	p-value
N	12	14	NS
Age (years)	61.67 ± 9.1	63.21 ± 12.69	NS
Male	9 (75%)	7 (50.00%)	NS
Somatometrics:			
Height (cm)	172.60 ± 8.26	160.00 ± 14.14	NS
Weight	80.30 ± 10.66	68.50 ± 23.33	NS
BMI	26.59 ± 3.13	26.82 ± 3.52	NS
Smoker	7 (43%)	5 (35%)	NS
Medical history:			
MI	6 (50%)	5 (35.0%)	NS
PCI	4 (33.3%)	4 (28.5%)	NS
CABG	2 (16%)	1 (7%)	NS
Hypertension	9 (75%)	9 (64%)	NS
Diabetes	5 (41.6.%)	6 (42.8%)	NS
Dyslipidemia	9 (75%)	9 (64.2%)	NS
Echocardiographic indices:			
LVEF(%)	49.3 ± 15.7	53.7 ± 12.99	NS
LVH	5 (41.6%)	8 (57%)	NS
LAd (cm)	38.48 ± 10.23	41.69 ± 8.71	NS
E (cm/s)	76.31 ± 29.51	72.3 ± 3.81	NS
A (cm/s)	67.08 ± 37.7	91.5 ± 20.5	NS
DT (s)	226.75 ± 95.7	246 ± 15.5	NS
S (cm/s)	7.48 ± 2.47	9.21 ± 2.9	NS
E´ (cm/s)	8.03 ± 1.98	9.3 ± 0.89	NS
A´ (cm/s)	9.93 ± 4.46	12.42 ± 3.64	NS
E/E´	9.65 ± 3.69	7.82 ± 1.15	NS

DSE – dobutamine stress echocardiography; BMI – body mass index; MI – myocardial infarction; PCI – percutaneous coronoary intervention; CABG – coronary artery bypass grafting; LVEF – left ventricular ejection fraction; LVH – left ventricular hypertrophy; LAd – left atrial diameter; DT – deceleration time; E – pulse wave Doppler early left ventricular filling velocity; A – pulse wave Doppler late left ventricular filling velocity; S, E´, A´ – tissue Doppler imaging systolic and early and late diastolic velocities of mitral annulus, respectively.

Table 2. Changes in sICAM-1 and sVCAM-1 levels in the DSE positive and DSE negative groups from baseline to peak stress.

		sICAM-1			sVCAM-1		
		Baseline ng/mL	Peak ng/mL	p	Baseline ng/mL	Peak ng/mL	p
Group A DSE(+) & CAD (+) n=12	Mean ± SD	302.57 ± 43.37	322.07 ± 49.64	0.043	781.48 ± 198.87	790.23 ± 231.43	0.699
Group B DSE (-) n=14	Mean ± SD	267.47 ± 28.03	260.43 ± 36.45	0.148	797.09 ± 277.16	787.89 ± 253.15	0.555
p		0.028*	0.001*		0.872	0.981	

DSE – Dobutamine stress echocardiography; CAD – coronary artery disease; sICAM-1 – soluble ICAM-1; sVCAM-1– soluble VCAM-1. *p<0.05

ole, plasma levels of sICAM-1 increased significantly in patients with ischemic heart disease, whereas they remained unchanged in patients with syndrome X and in the control group.[21] This was attributed to the "coronary steal" phenomenon caused by dipyridamole, which may result in endothelial cell activation and inflammatory response, coordinated by a number of inflammatory cytokines that upregulate the expression of leukocyte-endothelial adhesion molecules such as ICAM-1.[22-24] That study also reported significantly decreased plasma levels of soluble VCAM-1 in patients with ischemic heart disease following the dipyridamole stress test, whereas they remained unchanged in patients with syndrome X, and in the control group.[21] The authors attributed this observed decrease to an inflammatory response caused by the myocardial ischemia, which intensifies cell-cell contact and subsequent "trapping" of the molecules.[25]

In contrast, our study showed that sVCAM-1 levels remained unchanged from baseline to peak stress

in both groups. That could be explained by the fact that our study was performed in a different group of patients, and additionally by the fact that dobutamine provokes ischemia mainly through the inotropic and chronotropic response stimulating the myocardial beta-1 receptors and causing an increase in myocardial oxygen demand, so "trapping" of the molecules is less likely to happen.[15,26-28] Nevertheless, a local inflammatory response could very well have a part to play in the sICAM-1 increase, as it has been reliably shown that inducible ischemia leads to cytokine production and tissue factor expression after DSE in patients with CAD.[29]

Our study shows a clear correlation between sICAM-1 kinetics and DSE results. This correlation could possibly be utilized in several clinical scenarios, where there is suspicion of CAD but inducible ischemia testing cannot be performed or is unavailable. sICAM-1 levels could also form part of a diagnostic algorithm in cases of ambiguous DSE results.

Future studies could further examine the relation between sICAM-1 and DSE testing, especially whether sICAM-1 kinetics have any prognostic value in the setting of a positive DSE. Another interesting perspective for a future study would be the implementation of a virtual histology coronary intravascular ultrasound examination in order to provide additional data concerning the plaque structure and grade of inflammation that could be correlated with the levels of adhesion molecules.

Study limitations

The relatively small study population from only one center was one of the limitations of this study, as a greater population might have strengthened the observed differences in a statistically significant manner. Another possible limitation is the lack of angiographic data from the DSE negative group that could show whether coronary artery disease was present despite a negative DSE response.

Conclusions

The most significant finding in this study was the elevation of sICAM-1 plasma levels in subjects with a positive DSE and CAD, as determined by coronary angiography, compared to those with a negative DSE. The angiographic data confirmed that most of these patients suffered from significant coronary artery disease. This could contribute to an improvement in the diagnostic accuracy of the method and could shed more light on the pathophysiology of ischemia in coronary heart disease.

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